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Sphingolipidoses

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■ *Sphingolipidoses are an heterogeneous group of inherited disorders of lipid metabolism affecting primarily the central nervous system. These disorders occur chiefly in the pediatric population, and the degenerative nature of the disease processes is generally characterized by diffuse and progressive involvement of neurones (gray matter) with psychomotor retardation and myoclonus or of fiber tracts (white matter) with weakness and spasticity.*

Biochemical research has identified the defects in the sphingolipidoses to specific lysosomal enzymes. For example, Niemann-Pick disease lacks sphingomyelinase; Krabbe's disease lacks galactocerebrosidase; Gaucher's disease lacks beta-D-glucosidase; metachromatic leukodystrophy lacks sulfatase; Tay-Sachs disease lacks hexosaminidase A; and generalized gangliosidosis lacks beta-galactosidase.

Although there are no currently available modes of rendering corrective therapy in these disorders, a definitive diagnosis is possible both antepartum as well as postpartum. This information provides a sound and accurate basis for genetic counseling.

THIS REVIEW WILL FOCUS upon recent advances in neurochemistry of the sphingolipidoses. The term sphingolipidoses refers to a group of inherited disorders of lipid metabolism affecting chiefly the nervous system. The clinical problems have stimulated a wealth of productive research in neurochemistry, particularly in the areas of brain lipids and membranes. These investigations have also provided a foundation for future research into the molecular basis of normal brain function and structure. This review will describe briefly the current neurochemical information regarding the following sphingolipidoses: Niemann-Pick disease, Krabbe's disease, Gaucher's disease, metachromatic leukodystrophy, Tay-Sachs disease, and generalized gangliosidosis.[1-4] (See Table 1.)

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Clinical Aspects

The sphingolipidoses are a heterogeneous group of diseases and the diagnosis on clinical grounds is oftentimes difficult. However, a clinico-pathological approach is possible with these as well as other so-called degenerative diseases of the central nervous system.

These diseases can be divided into those that have primarily gray matter symptoms and signs

TABLE 1.—*Sphingolipidoses*

			Lipid	*Enzyme Defect*	*Disease*
CERAMIDE+	—P-choline (phosphorylcholine)		=sphingomyelin	sphingomyelinase	Niemann-Pick**
	—Hexoses	—galactose	=galactocerebroside	galactocerebrosidase	Krabbe's
		—glucose	=glucocerebroside	β-D-glucosidase	Gaucher's
		—gal-SO_4	=sulfatide	sulfatase	M.L.D. (sulfatide lipidosis)
		—trihexose	=ceremide trihexoside	ceremide trihexosidase	Fabry's
		—hexoses+ NANA*	=gangliosides:		
			G_{M_2} ganglioside	Hexosaminidase A	Tay-Sachs***
			G_{M_1} ganglioside	β-galactosidase	Generalized gangliosidosis
		fucose		fucosidase	Fucosidosis
	CERAMIDE=	sphingosine + fatty acid	palmitic acid + serine		

*NANA=N-acetylneuraminic acid
**There are 4 clinically separate forms of Niemann-Pick and only type A is referred to above.
***There are now 5 gangliosidoses with varying ganglioside and lysosomal enzyme deficiencies.

and those with primarily white matter symptoms. With the former, the gray matter symptoms and signs are those of either "irritation," such as seizures, particularly myoclonic seizures, or "inhibition," such as apathy, lethargy and dementia. Further, if occipital neurons are involved, cortical blindness will supervene. With diseases affecting primarily the white matter, the symptoms and signs are those of long-tract involvement; for example, spastic weakness with involvement of the corticospinal tract, pseudobulbar palsy with damage to the corticobulbar tract, incoordination from destruction of cerebellofugal fibers, and even cortical blindness, secondary to interruption of the optic radiations. Tay-Sachs disease would be a prototype of a disease affecting primarily gray matter, while metachromatic leukodystrophy would be more typical of disease predominantly of white matter.

Niemann-Pick Disease

Niemann-Pick disease[5-7] is a genetic disorder subdivided into four groups depending upon the age of onset and clinical manifestations. The infantile form or Crocker's type A accounts for approximately 85 percent of the cases of Niemann-Pick disease and definitive lipid abnormalities in brain are limited to this subgroup. The infantile form of Niemann-Pick disease is characterized clinically by psychomotor retardation with mental deterioration, seizures, spasticity, hepatosplenomegaly and frequently the presence of a "cherry red spot" in the fundi. (The "cherry red spot" is seen in several of the lipid storage diseases and is not diagnostic of any one entity.)

Pathological examination of enlarged visceral organs, brain and bone marrow discloses the presence of "foam cells" laden with lipid material. Analysis of the lipid reveals an abundance of sphingomyelin. An enzymatic mechanism that accounts for the abnormal intracellular deposition of sphingomyelin—that is, excessive synthesis or inadequate breakdown—was established by the discovery that patients with this disease lacked the hydrolytic enzyme sphingomyelinase.[6] As with other similar disorders, it is anticipated

that future research will provide a rational means by which to "induce" the deficient enzyme or prevent the abnormal accumulation of the lipid.[4]

Krabbe's Disease

Krabbe's disease[8-12] is an autosomal, recessive, genetic disorder affecting primarily the central nervous system. Following normal early development, the disease has its onset during the middle of the first year of life. Symptoms are those of psychomotor retardation, tonic seizures, spasticity and blindness. Recently peripheral neuropathy has been described.

Histological examination of brains disclose diffuse demyelination of white matter with arcuate fiber sparing. In addition, there is the characteristic accumulation of large epithelioid or "globoid" cells in the white matter and hence its noneponymic title of "globoid cell leukodystrophy."

Lipid analyses of brains affected with Krabbe's disease have given conflicting results, but separation and analysis of globoid cells indicate a higher relative concentration of cerebroside. This cerebroside's sugar moiety is galactose and hence is termed galactocerebroside. The proposal that a focal accumulation of galactocerebroside may account for the disease is supported by experimental studies involving the intracerebral injection of galactocerebroside. Dogs injected in this fashion developed neurological deficits associated with typical epithelioid or globoid cells in white matter.

A postulated enzyme defect in this disorder is unique, because it is in the synthetic as opposed to the more common catabolic pathway. The deficient enzyme is the cytoplasmic cerebroside, sulfotransferase. This enzyme transfers sulfate from the donor PAPS (phosphoadenosinephosphosulfate) to the 3-carbon of the galactose molecule of cerebroside. A genetic disorder in dogs manifesting similar histological and biochemical alterations as globoid cell leukodystrophy should help to clarify the molecular pathogenesis of this disease and offer a laboratory model for possible means of therapy. Recently, however, Suzuki[12] has accumulated compelling evidence in human brain tissue that the metabolic defect in Krabbe's disease is in fact a catabolic enzyme, namely, the lysosomal galactocerebroside-β – galactocerebrosidase.

Gaucher's Disease

Gaucher's disease[13-15] is an autosomal, recessive genetic disorder that has its onset in infancy or adulthood and infrequently during adolescence. The infantile form characteristically affects the central nervous system and is sometimes referred to as the "cerebral form" of Gaucher's disease. Clinically this disease is similar to Niemann-Pick disease in its presentation with psychomotor retardation, poor feeding due to bulbar weakness, opisthotonus, spasticity, and striking hepatosplenomegaly. Death ensues in the first year of life in a decerebrate state.

Histologically affected tissues contain large, multinucleated cells with a characteristic dull, waxy appearance. The lipid accumulation is due to cerebroside. Since the sugar moiety is glucose, it is designated a glucocerebroside.

Investigations for an enzyme defect have revealed an absence of lysosomal beta-D-glucosidase, essential for the normal degradation of glucocerebrosides.

Metachromatic Leukodystrophy

Metachromatic leukodystrophy[16-21] (MLD or sulfatide lipidosis) is an autosomal, recessive, genetic disorder known since 1910 that becomes manifest during childhood and occasionally in adulthood. The symptoms in childhood are those of leukodystrophy, in that motor symptoms predominate. Muscular weakness and wasting, associated with a stumbling gait or *genu recurvatum*, may be the initial symptom.

MLD is unique among the sphingolipidoses because the peripheral nervous system is affected. Thus, evidence of a peripheral neuropathy with sensory, motor, and reflex changes may precede or supersede the findings of a leukodystrophy. With progression of the disease, generalized cerebral dysfunction develops. Seizures occur in one-half the patients, blindness in one-third, and death follows a decerebrate or decorticate state. The adult form of MLD usually presents as an organic dementia or psychosis, frequently diagnosed as schizophrenia. These patients, however, eventually manifest diffuse central nervous system involvement with corticospinal, corticobulbar, and cerebellar symptoms.

Histochemical studies disclose the presence of intracellular lipids, not only in the central and peripheral nervous systems, but also within visceral organs, such as kidney, liver, and gallblad-

der. Impairment of these organs does not become clinically apparent. Characteristic staining is metachromasia—that is, an alternate color to the dye used, such as toluidine blue staining red or cresyl violet staining brown. In addition to the deposition of sulfatide within white matter cells, there is symmetrical demyelination of white matter with U-fiber or arcuate fiber sparing. Intraneuronal collection of metachromasia is not characteristic except for few Betz cells and for certain subcortical neurones, notably the cerebellar dentate nucleus.

Identification of sulfatide as the lipid responsible for metachromasia in MLD was first demonstrated in 1958 by two independent workers, Austin in the United States and Jatzkewitz in Germany. Lipid analyses in MLD reveal a ten-fold increase in brain sulfatide concentration with an inversion of the sulfatide: cerebroside ratio to 4:1. Normally the ratio is approximately 0.25 to 1. Analysis of the lipid composition of myelin in MLD demonstrates a preponderance of sulfatide; this has led to the speculation that demyelination in this disease may be due to formation of an unstable membrane. O'Brien reasoned that the preponderance of sulfatide in the myelin membrane could lead to a less cohesive membrane because of abnormalities in the surface charge of the membrane, due to an excess of electronegatively charged sulfate groups. MLD appears to be one of the first examples of a disease resulting from a molecular defect in membrane structure.[18]

Although the clinical diagnosis of MLD is suggested by the presence of leukodystrophic symptoms and signs combined with a peripheral neuropathy, the definitive diagnosis is established by one of several techniques. The older technique of demonstrating metachromatic granules in the urine with toluidine blue can give false-positive results, since other substances besides sulfatides (mucopolysaccharides, for example), are metachromatic. A sensitive and accurate test devised by Austin is detection of arylsulfatase in urine. Affected persons have no enzyme activity. Alternatively, biopsy of a nerve, such as sural nerve, with demonstration of metachromasia would establish the diagnosis of MLD. Biochemical studies on tissues from MLD have identified an absence of sulfatase, the lysosomal enzyme which hydrolyses the sulfate group from sulfatide. Clinical investigations of sulfated compound reveal a slow turnover in MLD compared to normal subjects. These findings would support *in vitro* biochemical data of defective catabolism of sulfatide.

Attempts to restrict sulfur intake and thereby the available precursor pool for sulfatide synthesis or the infusion of the missing enzyme sulfatase have been unsuccessful in altering progression of the disease in clinical trials. Knowledge that vitamin A is a required cofactor in the synthesis of PAPS (phosphoadenosinephosphosulfate) has raised the possibility of inducing vitamin A deficiency in MLD to decrease the available activated sulfate for sulfatide synthesis.

Clinical application of this proposal would appear to have impracticalities, but information to date is insufficient.

Tay-Sachs Disease

Tay-Sachs disease[22-28] or infantile amaurotic familial idiocy (AFI) is a genetic disease occurring primarily in Jewish infants. The disorder commences during the middle of the first year of life after an apparently normal early development. Death ensues after a period of two to four years. The brunt of the disease is upon the central nervous system, particularly the grey matter. As the name implies, amaurosis develops, and the so-called "cherry-red spot" of the macular region is to be seen on funduscopic examination and idiocy is concomitant. In addition, grey matter involvement gives rise to a cortical irritative phenomenon, manifested by myoclonic seizures. Pathological examination reveals "ballooned" neurones filled with lipid staining material. Electron microscopic studies of the involved neurones disclose the cytoplasm to be filled with membranous, lamellated bodies termed "membranous cytoplasmic bodies" or MCBs. Lipid analysis of these bodies, obtained by differential centrifugation, reveals an increase of monosialoganglioside. This ganglioside which lacks the terminal galactose is normally found in very small quantities and is referred to as the "Tay-Sachs ganglioside" or G_{M_2} ganglioside (Svennerholm's nomenclature). The enzymatic defect in Tay-Sachs disease is now known to be an absence of a lysosomal beta-D-N-acetylhexosaminidase.[8] An as yet unexplained and unconfirmed finding in Tay-Sachs disease is decreased activity of serum fructose-1-phosphate-aldolase.[23] Recently, O'Brien and Okada have taken amniotic fluid of suspected carrier mothers and applied tissue culture techniques to grow fibroblasts upon which enzyme

TABLE 2.—*Diagnostic Tests for Sphingolipidosis*

Disease	*Blood*	*Tissues*	*Other*
Laboratory Diagnosis			
Niemann-Pick	vacuolated lymphocytes	foam cells in marrow, involved viscera	
Krabbe's		brain* and peripheral nerve biopsy	elevated CSF protein
Gaucher's	deficient Beta-glucosidase in WBC or skin	Gaucher's cells in marrow and involved viscera	
MLD (sulfatide lipidosis)	decreased WBC sulfatase	peripheral nerve biopsy showing metachromasia	urinary sulfatide increase or sulfatase decrease
Tay-Sachs	absent hexosaminidase A in serum & WBC	rectal and/or brain* biopsy; fibroblast from skin; prenatally by amniotic fluid cell assay	elevated CSF protein
Generalized gangliosidosis	deficient beta-galactosidase in WBC	brain* and/or visceral biopsy; foam cells in marrow	deficient beta-galactosidase in urine

**Brain Biopsy.* Establishment of a definitive diagnosis with the use of brain biopsy is justified for genetic counseling provided the patient demonstrates irreversible dementia and that adequate neurosurgical, histological and biochemical facilities are available.

analyses are made. The diagnosis of Tay-Sachs disease may be possible before birth by finding a deficiency of hexosaminidase.[25] This technique of amniocentesis holds great promise as a powerful diagnostic tool and opens doors for its utilization for antepartum diagnosis in other genetic disorders.[26,28]

Generalized Gangliosidosis

Generalized gangliosidosis (or G_{M_1} gangliosidosis, type 1)[29-31] is an acute infantile disease characterized by psychomotor retardation, hepatosplenomegaly, and coarse features similar to those of Hurler's disease. Generalized gangliosidosis is due to an accumulation of G_{M_1} ganglioside in brain and viscera as well as mucopolysaccharide in the latter. Death ensues in the first two years of life. The disease has been confused with Tay-Sachs disease because of the finding of a cherry red spot in the macula and psychomotor retardation, with Hurler's disease because of the presence of similar phenotypic abnormalities. Enzymic studies disclose a pronounced deficiency of beta-galactosidase which accounts for accumulation of the G_{M_1} ganglioside and the mucopolysaccharide. There is as yet no specific form of therapy but early recognition and diagnosis is important so that accurate genetic counseling can prevent similar births.

Juvenile G_{M_1} gangliosidosis (or G_{M_1} gangliosidosis, type 2) has as its onset about age one and is due to the cerebral but not visceral accumulation of G_{M_1} ganglioside. Death ensues within three to ten years. Although beta-galactosidase is also absent in this disease, it is phenotypically distinct from generalized gangliosidosis because of the absence of visual disturbances, cherry red spot of the macula, hepatosplenomegaly, and significant bony deformities. (Late infantile amaurotic idiocy or Jansky-Bielschowsky disease is phenotypically distinct from generalized gangliosidosis and does not represent a sphingolipidosis.)

Table 2 shows diagnostic tests for the identification of various kinds of sphingolipidoses.

Neurochemistry of Brain Lipids

The brain is 80 percent water, yet of the dry weight, brain lipids are the major constituent (60 percent and are classified into three main groups: (1) the sterols, primarily cholesterol, (2) the phospholipids, and (3) the sphingolipids. The major types of sphingolipids are gangliosides, cerebrosides, sulfatides, and sphingomyelin. Sphingolipids constitute approximately 10 percent of whole brain lipids and approximately 20 percent of purified myelin lipids. Of interest is the localization of gangliosides in neurons and their virtual absence in myelin.

The sphingosine molecule is an essential constituent for the structure of sphingolipids from which it derives its name. Sphingosine is synthesized from the condensation and subsequent decarboxylation of palmitic acid and serine to form an 18-carbon amino-sugar. The three main groups of lipids noted above of cholesterol, phospholipids, and sphingolipids are assembled with pro-

tein moieties to form membranes and become the structural boundary of cells and their subcellular organelles. A postulated structure of membranes is that of a "bimolecular leaflet" with interdigitation of opposing lipid molecules. This concept was derived from electron-microscopic and x-ray diffraction data.

Anatomically, gangliosides are associated primarily with neurons, or "ganglion" cells, from which they derive their name. This highly polar lipid plays an important role in neuronal function, but its precise nature is as yet unknown. Subcellular fractionation of nerve cells has yielded information that gangliosides are associated primarily with microsomes and synaptosomes. Gangliosides are distinct from other sphingolipids by containing one or more N-Acetylneuraminic acid (NANA) molecules, the biologically most significant member of the family of sugars called sialic acid. Ten separate gangliosides have so far been identified, depending on the number of attached NANAS (1 to 3), their combination, and the number of attached hexoses. Over 90 percent of the gangliosides are accounted for by four major gangliosides: one monosialoganglioside (different from the Tay-Sachs ganglioside which lacks a terminal galactose), two disialogangliosides, and one trisialoganglioside. The complex nature of gangliosides is suggested by their dependence upon protein synthesis, since ganglioside synthesis is inhibited by puromycin, an inhibitor of protein synthesis.

Although sphingomyelin contains the word "myelin," it is not found exclusively nor abundantly in myelin and, therefore, is a misnomer. Cerebroside, on the other hand, is found in relative abundance in myelin and is frequently considered a "marker" of myelin lipids.

Normally, myelin contains three to four times as much cerebroside as sulfatide. Sulfatide is a sulfate ester of cerebroside and is synthesized by the addition of sulfate to cerebroside from the activated sulfate, phosphoadenosine-phosphosulfate (PAPAS), by the cytoplasmic enzyme, cerebroside sulfotransferase.

This brief review has summarized the current knowledge of the biochemical abnormalities in the sphingolipidoses. Elucidation of this heterogeneous group of rare diseases of the central nervous system has followed the traditional sequence of clinical characterization, pathological verification, chemical analysis, and finally the biochemical identification of the enzymatic defect. These "experiments of nature" have provided a biochemical insight into the complexity of the nervous system. It is anticipated that continued exploration of these and similar disorders of brain will yield rational clues to their therapy as well as shed light on the molecular basis of normal neural function.

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